Fibre digestibility in large herbivores as related to digestion type and body mass — An in vitro approach

Steuer, Patrick ; Südekum, Karl-Heinz ; Müller, Dennis W H ; Kaandorp, Jacques ; Clauss, Marcus ; Hummel, Jürgen

Abstract: The coexistence of different ungulate species in a given ecosystem has been the focus of many studies. Differences between ruminant foregut fermenters and hindgut fermenters were remarkable for example in the way they ingest and digest high fibre diets. Digestion trials based on total collections are difficult to conduct or are sometimes even not possible for wild animals in the field or in zoos. To gain information on the fibre digestion achieved by these animals and the influence of body mass (BM) thereon, a method using spot sampling is desirable. In this study, in vitro fermentation of faecal neutral detergent fibre (NDF) was used as a measure of fibre digestion in large ungulates. Food and faecal samples of 10 ruminant foregut fermenting and 7 hindgut fermenting species/breeds were collected. All animals received 100% grass hay with ad libitum access. The NDF of food and faeces was fermented in vitro in a Hohenheim gas test (HGT) for 96 h. The digestion type generally had an effect on the gas production (GP) of faecal NDF in the HGT with hindgut fermenters showing higher values than ruminant foregut fermenters. At any time interval of incubation, BM had no influence on GP. The results are in accordance with both findings that ruminant foregut fermenters have longer mean retention times and more comprehensive particle reduction and findings of a lack of influence of BM on digesta mean retention time. It can be stated that the HGT (96 h) is a useful and quick method to show also small differences within groups in fibre digestion.

DOI: https://doi.org/10.1016/j.cbpa.2012.10.026

Fibre digestibility in large herbivores as related to digestion type and body mass - an *in vitro* approach

Patrick Steuer[a,*], Karl-Heinz Südekum[a], Dennis W. H. Müller[b,d], Jacques Kaandorp[c] Marcus Clauss[b], Jürgen Hummel[a,e]

[a]*Institute of Animal Science, University of Bonn, Germany*

[b]*Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Switzerland*

[c]*Safari Park Beekse Bergen, Hilvarenbeek, The Netherlands*

[d]*National Park 'Bavarian Forest', Grafenau, Germany*

[e]*Department of Animal Sciences, University of Goettingen, Germany*

***Corresponding author:**

Patrick Steuer, Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany. Tel.: +49-228 73 60478; Fax: +49-228 73 2295; email: pste@uni-bonn.de.

Abstract

The coexistence of different ungulate species in a given ecosystem has been the focus of many studies. Differences between ruminant foregut fermenters and hindgut fermenters were remarkable for example in the way they ingest and digest high fibre diets. Digestion trials based on total collections are difficult to conduct or are sometimes even not possible for wild animals in the field or in zoos. To gain information on the fibre digestion achieved by these animals and the influence of body mass (BM) thereon, a method using spot sampling is desirable. In this study, *in vitro* fermentation of faecal neutral detergent fibre (NDF) was used as a measure of fibre digestion in large ungulates. Food and faecal samples of 10 ruminant foregut fermenting and 7 hindgut fermenting species/breeds were collected. All animals received 100% grass hay with *ad libitum* access. The NDF of food and faeces were fermented *in vitro* in a Hohenheim gas test (HGT) for 96 h. The digestion type generally had an effect on the gas production (GP) of faecal NDF in the HGT with hindgut fermenters showing higher values than ruminant foregut fermenters. At any time interval of incubation, BM had no influence on GP. The results are in accordance with both, findings that ruminant foregut fermenters have longer mean retention times and more comprehensive particle reduction, and with findings of a lack of influence of BM on digesta mean retention time. It can be stated that the HGT (96 h) is a useful and quick method to show also small differences within groups in fibre digestion.

Keywords: hindgut fermenters, Hohenheim gas test, ruminant foregut fermenters, spot sampling

1. Introduction

Among the major features allowing ungulates to coexist with several taxa in ecosystems is the way they make use of available food resources. Relevant characteristics in this respect are type of digestive tract, feeding type or body mass (BM) of a species (Owen-Smith, 1988; Van Soest, 1994). Such characteristics have the potential to influence variables such as diet quality (via feeding selectivity), capacity to handle ”mechanically tough” lignified/woody material, or digestibility realized by the species (Demment and Van Soest, 1985). Digestibility of fibre is generally considered to be of particular significance in this respect. There are two different strategies to handle high fibre diets:

a) to ingest a moderate amount of fibrous forage, ruminate the forage and have a long food mean retention time, and hence have a high fibre digestibility (strategy found in ruminant foregut fermenters);
b) to ingest large amounts of fibrous forage, not to ruminate the forage and have average to short food mean retention times, and hence have low fibre digestibilities (strategy mostly found in large hindgut fermenters).

Comprehensive evidence exists for superior fibre digestion in ruminants compared to hindgut fermenters in general (Foose, 1982; NDF digestibility in hindgut fermenters 44% vs. 59% in ruminant foregut fermenters). Within hindgut fermenters, there is also evidence that rhinoceroses (at least white, *Ceratotherium simum*, and the Indian, *Rhinoceros unicornis*) and equids are superior to tapirs and elephants in this respect (Foose, 1982; Clauss et al., 2005; Clauss et al., 2006). Concerning feeding types, there is indication for superior fibre digestion in grazers compared to browsers (ruminant foregut fermenter: Prins et al. (1983), van Wieren (1996), Iason und van Wieren (1999), Pérez-Barbería et al. (2004); rhinoceros: Clauss et al. (2006), Steuer et al. (2010)). However, evidence for an influence of BM on fibre digestibility is less clear: Robbins et al. (1995) drew a conclusion that there is a significant effect of BM on this trait; Van Soest et al. (1995) found a significant effect on digestibility of grass

75 cellulose, but not of lucerne cellulose and on hemicelluloses of both forage types. Van Wieren
76 (1996) and Iason and van Wieren (1999) reported some evidence for an influence of BM, but
77 at the same time stated that it is clearly limited. Others like Owen-Smith (1988), Wenninger
78 and Shipley (2000), Pérez-Barbería et al. (2004) and Clauss et al. (2009) did not support the
79 idea of a positive effect of BM on fibre digestibility.
80 For any quantitative exploration of the effect of particular characteristics on animal
81 performance, comprehensive comparative datasets are required. However, studies including a
82 considerable number of captive non-domestic specimens soon face inevitable restrictions
83 caused by a limitation to interfere with management practices in wild animal collections.
84 Methods not relying on quantitative collection of food intake and faeces production are
85 desirable. Methods using external markers like TiO_2 (Jagger et al., 1992; Kavanagh et al.,
86 2001; Titgemeyer et al., 2001; Glindemann et al., 2009) still require accurate quantification of
87 food intake in situations where marker intake is not necessarily proportional to intake of the
88 whole diet (as typical in herbivores), and internal markers like acid insoluble ash (AIA)
89 (Kavanagh et al., 2001) still require a degree of accuracy which is not always feasible (e.g.,
90 incidental ingestion of small amounts of sand renders AIA inapplicable).
91 Prins et al. (1981) used *in vitro* fermentation for measuring the indigestible neutral detergent
92 fibre content in food and spot samples of faeces of ponies and wethers. They calculated the
93 digestibility of the fraction using this information. Since the results were in agreement with a
94 parallel *in vivo* study, they concluded that this method can be used in cases where classical
95 digestion trials were not possible but stress the point that the diet has to be known. Another
96 option not relying on quantitative evaluation of faecal output or food intake and using *in vitro*
97 fermentation of fibre residues of faeces from uniformly fed animals was used by Steuer et al.
98 (2010). The principle is that digestion in the animal will be negatively correlated to *in vitro*
99 digestibility of residual fibre (quantified via gas production in this case); a high fibre

digestibility realised by the animal will result in a low digestibility of faecal fibre residues in the *in vitro* system (and vice versa).

In this study, the latter approach was used to investigate the influence of digestion type (ruminant foregut fermenter vs. hindgut fermenter) and BM on *in vitro* digestibility of faecal fibre in a sample of 17 domestic and non-domestic ungulate species. Digestion type has been shown to be related to fibre digestibility, which is generally higher in ruminants (*in vivo*). The *in vitro* test as used in this study should therefore yield higher gas production from faecal fibre of hindgut fermenters. In contrast to the influence of digestion type, an influence of BM on fibre digestibility via increased digesta retention times is still debated. Based on own results indicating no increase of retention time with BM *in vivo*, no decrease of gas production from faecal fibre with BM should occur *in vitro*.

2. Materials and Methods

2.1. Animals and feeding

Food and faecal samples of 10 ruminant foregut fermenters (9 ruminant and 1 camelid species, n per species = 2-6) and 7 hindgut fermenting species/breeds (n per species = 3-7, warthog n = 1) were collected (Table 1). Due to contamination with saw dust, which was used as litter in the stables, it was not possible to collect food left overs for all of those animals which were fed from hay racks. Trials were conducted in the winter seasons 2008 and 2009. The zoo animals were sampled at safari park Beekse Bergen in The Netherlands. Shetland ponies and sheep were sampled in Zurich (Vetsuisse Faculty Zurich/Swiss Federal Institute of Technology Zurich). Horses were sampled at a private riding stable, the goats and domestic cattle were sampled at the University of Bonn. All animals were adult and neither pregnant nor lactating during the trial. Exceptions were two of the sable antelopes, which were in the first stage of pregnancy (1-2 month).

124 Cattle, goats, sheep, horses, ponies and the warthog were weighed; BM of the other zoo
125 animals were estimated by zoo keepers, zoo veterinarians and the conductor of this study. All
126 animals received 100% grass hay with *ad libitum* access for an adaptation period of 14 days.
127 The grass hay was the second cut of a mixed sward. In general, animals were kept separately
128 during the collection period. Food and faecal samples (representing a large proportion of total
129 daily defecation) were collected daily for a minimum of 5 days after the adaptation period.
130 Food samples were pooled after the trial. All boxes and stables were covered with material the
131 animals did not feed on (saw dust, rubber mats). Most of the animals were fed from hay racks.
132 Some of them were fed using feeding troughs (Shetland pony, domestic cattle and domestic
133 horse) or from the ground (warthog and white rhinoceros). Exceptions for points mentioned
134 above were the African elephants (which had daily access to outside enclosure for 4-6 h as a
135 group), springboks, Przewalski horses and Bactrian camels. The latter two were permanently
136 kept on large outside enclosures, but food intake of these pastures was insignificant due to the
137 season (Jan. - Feb. 2008). The springboks were kept in individual enclosures, but because
138 they could not be shifted and faeces could not be collected with the animals in the enclosures,
139 food and faecal samples were collected once.

140 *2.2. Chemical analysis*

141 Faecal samples for chemical analysis were pooled at the end of the trial, stored at -20°C for
142 further analysis and later freeze dried. Grass hay and all faecal samples were ground through a
143 1 mm sieve (centrifugal mill, model ZM1, Retsch, Haan, Germany). Dry matter (DM) and ash
144 were analyzed according to VDLUFA[1] (2007; method 3.1, 8.1). Neutral detergent fibre
145 (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analyzed
146 sequentially for the grass hay and faeces according to Van Soest and Robertson (1985) with
147 the Gerhardt fibre-bag system (Gerhardt, Königswinter, Germany). The NDF and ADF were

[1] Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

corrected for ash using the insoluble ash after lignin determination. Nitrogen content was analysed according to VDLUFA (2007; method 4.1.2, Dumas). Digestibility of the grass hay fed was estimated using the standard 24 h Hohenheim gas test (HGT) (Menke and Huss, 1987) (see below for details on Hohenheim gas test analysis).

2.3. In vitro *fermentation*

The approach of this study was to use residual fermentability of fibre isolated from faeces as a proxy for the degree of fibre fermentation in the gut of the animal; the more fermentable the fibre left in faeces, the less it was digested in the gut of the animal. To isolate the fibre fraction, faeces and the respective grass hays were first boiled with neutral detergent (ND) solution according to Van Soest et al. (1991). *In vitro* fermentation of these residues was evaluated with the Hohenheim gas test (HGT) (Menke et al., 1979) (3 replicates per individual; due to long fermentation times (see below), NH_4CO_3 was increased from 4 g/L to 6 g/L, and correspondingly $NaCO_3$ decreased from 35 g/L to 33 g/L in the buffer solution as suggested by Liu et al. (2002)). The test uses GP from the substrate as measure for digestibility (in this study expressed as ml/200 mg NDF; NDF expressed without residual ash). For all incubations, standardized mixed rumen fluid from 2 sheep on a diet of 50% grass hay and 50% compound concentrate at maintenance level was used. Gas production was quantified at 4, 8, 12, 16, 24, 32, 48, 56, 72, 80 and 96 h. The following fermentation intervals were finally evaluated: 0-8 h, 8-16 h, 16-24 h, 24-48 h, 48-72 h, 72-96 h. The studied animals had an average digesta mean retention time (MRT) of 55 h (Steuer et al., 2011) with a range of 17 - 78 h; 96 h was therefore chosen as a time safely covering potential differences between the species investigated. A HGT with considerably longer fermentation time (192 h) on samples of a ruminant and a hindgut fermenter (sheep and Shetland pony) supported the selection of appropriate maximal fermentation times (96 h) for the bulk of samples.

172 *2.4. Statistics*

173 All statistical comparisons were performed with species means. In order to account for
174 ancestry-based correlations in the datasets (i.e., finding a significant result simply because
175 similar species are closely related; Felsenstein (1985), Pagel (1999)), the data was controlled
176 for phylogenetic influences using the "Phylogenetic Generalized Least-Squares" method
177 (PGLS; Martins and Hansen, 1997; Rohlf, 2001). This procedure estimates a covariance
178 matrix of the species due to their ancestral roots and includes these interrelationships in a
179 generalized least squares algorithm to determine the model parameters. The phylogenetic
180 trees for the two datasets were derived by pruning the mammalian supertree from Bininda-
181 Emonds et al. (2007) to include only the species of concern for our study, using Mesquite
182 (Maddison and Maddison, 2006). The two different domestic horse breeds were represented
183 as direct relatives in the tree. Because the resulting trees were not based on our own
184 calculations of branch lengths with consistently the same characters, we used trees without
185 branch lengths. Statistical analyses were performed using ordinary least squares (OLS), which
186 did not account for phylogeny and using phylogenetic least squares (PGLS).
187 Statistical data evaluation (dependent variables: cumulative 96 h GP (96 h GP_{cum}), GP in
188 intervals 0-8 h, 8-16 h, 16-24 h, 24-48 h, 48-72 h and 72-96 h, and faecal NDF (FNDF)) was
189 done via analysis of variance using the linear model:
190
191 $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}$
192 where
193 Y_{ijk} = the observed response (dependant variable);
194 μ = the population constant, common to all observations;
195 α_i = the effect of BM (continuous variable);
196 β_j = the effect of digestion type j; j = 1-2 (hindgut fermenter or ruminant);
197 γ_k = 24 h GP of grass hay batches used in the respective trials (co-variable);

198 ε_{ijk} = the residual error.

199

200 For testing the effect of BM in the ANOVA, traditional allometric regression ($y = a + BM^b$)
201 was done for cumulative 96 h GP. To achieve normal distribution, data on BM were log-
202 transformed. Therefore, linear regression analysis of log-transformed measurements was used
203 for the estimation of allometries.
204 The statistical calculations were performed with PASW 18.0 (SPSS Inc., Chicago, IL) and
205 COMPARE 4.6 (Martins, 2004). The significance level was set to $\alpha = 0.05$. The 95%
206 confidence intervals were calculated for coefficients in allometric regressions.

207 # 3. Results

208 The mean NDF content of the fed grass hay was 72 ±3.7% in organic matter (OM); ADF
209 content was 39 ±3.9% OM, ADL content was 5.3 ±1.50% OM and for crude protein (CP) 10
210 ±1.8% OM was measured. The mean 24 h gas production (GP) for the grass hay was 33.1
211 ±4.21 ml/200 mg OM. There were no significant differences between the grass hay fed to
212 ruminant foregut fermenters and hindgut fermenters in NDF, ADF, ADL and CP content and
213 in 24 h GP (Table 1).
214 In an exploratory long-term (192 h) HGT run for the grass hay and the faecal fibre of sheep
215 and Shetland ponies, it was evident that gas production continued beyond the final
216 measurement (Fig. 1). However, differences between sheep and ponies were evident for the
217 interval 16-48 h and afterwards the differences stayed the same.
218 In general, results of OLS and PGLS data did not differ, and only the latter are discussed in
219 the following. Digestion type had an effect on the GP of faecal NDF residues, with hindgut
220 fermenters showing higher values (with the exception of time interval 8-16 h; PGLS: $p =$
221 0.156) (Table 2, 3; Fig. 2). The cumulative GP after 96 h of incubation and the FNDF content
222 were also lower for ruminant foregut fermenters compared to hindgut fermenters (Tables 2

223 and 3). The slight differences in the quality of the different batches of grass hay used (Table
224 1) did not have a significant effect on the results (Table 3). The closest it approaches to
225 significance was $p = 0.067$ for the interval 16-24 h.
226 No influence of BM was found for GP in time intervals, 96 h GP_{cum} and FNDF content.
227 If data of 96 h GP_{cum} were used to establish allometric regressions, in agreement with results
228 of the ANOVA allometric exponents were not different from 0 for the total data and within
229 digestion types (Table 4).

230 # 4. Discussion

231 *4.1. Differences between ruminant foregut fermenter and hindgut fermenters*

232 During the last decades, comparative digestive capacity has been in the centre of many
233 discussions on nutritional ecology of herbivores. Any investigation on this topic relies on
234 comprehensive datasets, which are hard to collect if food intake and faecal output have to be
235 measured quantitatively. The indirect method applied in this study has much less
236 prerequisites, relying only on a defined general feeding regime and faecal spot samples after
237 an appropriate adaptation period.
238 Differences in fibre digestion between digestion types such as ruminant foregut fermenters
239 and hindgut fermenters represented the first question that was approached with this method.
240 Since the basic differences between herbivores (ruminant foregut fermenters vs. hindgut
241 fermenters) are sufficiently well established in scientific literature, this also represents an
242 opportunity to confirm the validity of the method, which is desirable even if its principle is as
243 straightforward as in this case.
244 The study of Foose (1982) still represents the best single dataset quantifying fibre digestion
245 capacity in large herbivores. When applying a statistical approach identical to that used for
246 data of this study to the Foose (1982) dataset (OLS and PGLS approach; independent factors:
247 BM and digestion type, plus co-factor ADL-content of food), digestion type is a significant

248 factor for fibre digestibility in grass hay (Table 5). In consequence the expectation for the *in*
249 *vitro* trial clearly was to find a significant effect of digestion type on fibre digestibility,
250 particularly at later time intervals. The results clearly show the expected difference for all
251 time intervals except the second earliest (8-16 h), and it can be stated that the higher fibre
252 digestibility in ruminants is clearly reflected in the data of faecal fibre *in vitro* fermentability.
253 This is in line with their longer MRT compared to hindgut fermenters, which have also been
254 found in data of retention times for most of the animals of this trial (see Steuer et al. 2011:
255 MRT for small particles: ruminants 55 ±9.5 h, hindgut fermenters 34 ±9.1 h), and in their
256 higher degree of ingesta particle size reduction (Fritz et al., 2009).
257 As already mentioned by Foose (1982), further differences in strategies within hindgut
258 fermenters can be found e.g. for elephants vs. equids (Clauss et al., 2003) and even within
259 rhinoceroses (Clauss et al., 2006; Steuer et al., 2010). Compared to the difference between
260 digestion types, such differences are likely to require a higher resolution level and larger
261 sample sizes to be detected. Reference values from the Foose (1982) data are NDF-
262 digestibilities of 43% for the African elephant, 45% for the equids (with a range of 42-46%)
263 and 48% for the white rhino.
264 In a numerical comparison of values (given the scarcity of taxa, a sound statistical approach
265 appears difficult), our data are clearly in line with the position of the African elephant, which
266 as expected shows higher residual GP from faecal fibre than any other herbivore for the time
267 interval 8-16 h and 16-24 h. On the other hand, the *in vitro* data do not reflect a clearly higher
268 fibre digestibility for the white rhino compared to the average for equids, which is in line with
269 conclusions of Kiefer (2002), considering equids as a suitable nutritional model for the white
270 rhino.
271 Mean retention time has already been mentioned as a major determinant of fibre digestion.
272 Based on MRT alone, a superior role of rhinos among hindgut fermenters would be given
273 according to their longer MRT (Clauss et al., 2006; Steuer et al., 2011). However, differences

in digesta particle size (being negatively correlated to fermentation rate; Bjorndal et al. (1990)) must also be considered when explaining differences in fibre digestion. Fritz et al. (2009) gave an extensive overview on faecal particle sizes in herbivores, resulting in a ranking of elephants + rhinos > horses > ruminant foregut fermenters. This explains why a difference in fibre digestion between elephants (large particles and short MRT) and other hindgut fermenters of this study is unequivocal, while the difference between the white rhino (long MRT, but large particles) and equids (medium-sized particles, but short MRT) is less evident. Actually, the high chewing efficiency achieved by equids due to their intricate molar surface design might be a reason why they manage to co-exist within the same body size range as ruminant herbivores.

In conclusion, our method of estimating fibre digestibility via residual fermentability of faecal fibre largely supports expectations based on *in vivo* trials and does not present implausible results at any point (Foose, 1982).

Residual digestibility of faecal fibre has been used as a proxy for fibre digestibility in this study. The chemical basis for changes of *in vitro* digestibility of fibre from food to faeces are shifts in the proportion of fibre fractions in total fibre, in particular the accumulation of indigestible fibre fractions like lignin/ADL in total fibre (NDF). In fact, ADL content of faecal fibre (NDF) was higher in ruminant foregut fermenters (11.6% ±2.27) than in hindgut fermenters (8.8% ±1.19). Accordingly ruminant foregut fermenters digested more of the NDF and ADF fraction than hindgut fermenters.

4.2. Methodological considerations

As with any methodological approach, particular benefits and shortcomings are linked to its application. Its reliance on spot samples makes the method particularly attractive for comparative studies. What must be kept in mind is that a uniform diet still is an indispensible precondition for its application. This holds true even if an approach as in Prins et al. (1983)

would be chosen. In that study, fibre digestibility was related to theoretically digestible fibre rather than to total fibre (excluding the non-digestible fibre). While this was likely done with the intention to cope with the vast variation present among diets in the study (animals were investigated on their routine diets in 4 different zoos), differences in fermentation rate of the feedstuffs/diets are not accounted for by this correction. Such differences can be very prominent, e.g. between grass and lucerne (Smith et al., 1972), and are decisive for the digestibility as finally realised by the herbivore. Additionally, differences related to animal adaptations, such as the different digestive strategies, are most likely to be detected on challenging diets. This is evident when comparing results from grass-only diets between different species either in this study (Table 3) or in the data from Foose (1982, Table 5), where slight differences in diet quality did not influence the overriding effect of digestion type, to the results gained by Foose (1982) on lucerne hay. The lucerne hay generally had a lower fibre content, was distinctively better digestible, and differences in digestibility between species were explained by slight variations in the content of lignin (ADL) rather than by different digestive strategies (Table 5).

What may come surprising is that there is already some difference detectable for the earliest time interval (0-8 h), where only rather fast-fermenting and therefore easily digestible components are covered. Fast-fermentable fibre should be digested more or less completely in the animal´s gut already. The most likely explanation for the occurrence of significant residual digestion in the earliest time interval is that it represents a result of the necessary milling of the sample before analysis. This is likely to cause a very small particle fraction, whose largely increased surface/volume ratio enhances access and digestion of these particles by microbes, largely suspending the delay in fermentation typical for this material (lag-time). It should be stressed that for the method as applied in this study (quantifying *in vivo* fibre digestion via residual *in vitro* digestion in faecal fibre), it is an inevitable prerequisite that

faecal particles are brought to a uniform size (milling; 1 mm sieve) before *in vitro* fermentation.

As stated above, values from faeces with indicative value for digestive capacity bear promise for comparative studies. Besides residual GP from faecal fibre, total fibre content in faecal DM (FNDF) was included in this study. Although conclusions based on this value are largely equivalent to those from residual fibre digestibility (no influence of BM, influence of digestion type, Table 3), a major shortcoming may discourage its general use: Since it represents a concentration related to dry matter, its value will not only be depending on fibre (NDF), but also on dry matter digestibility.

4.3. Influence of body mass

Although several studies have approached the question, the relation of BM and fibre digestibility still represents a bone of contention. Based on the Jarman-Bell principle (JBP) (superior digestive capacity in large herbivores due to a difference in scaling of gut volume ($BM^{1.0}$) and energy requirements ($BM^{0.75}$)), an increase of MRT with BM is postulated. In consequence this would lead to an increased capacity for fibre digestion (particle size is rarely accounted for in such considerations). In fact, analyses can arrive at different conclusions (Table 6) even if they largely rely on a common data base (Foose (1982)).

Our own re-analyses of the Foose (1982) dataset (as outlined above; Table 5) support the lack of an influence of BM on digestion of NDF in grass hay. However, at least a trend was observed in phylogenetically controlled Foose (1982) data (PGLS: $p = 0.099$). Data on residual fibre digestibility of this study represent a valuable opportunity to approach the question with a comprehensive but completely independent dataset. The result was unequivocal: No relationship was found between BM and GP (in time intervals and cumulative 96 h) in the linear model (Table 3), and also not if investigated via classic allometric regression for 96 h GP_{cum}. This is in line with the general lack of an influence of

BM on MRT as found in Steuer et al. (2011) for data originating from largely the same data collection. Based on our results, it can be concluded that within the BM range investigated (representing the largest part of the range available in ungulates, except those < 30 kg), no significant influence of BM on fibre digestibility can be detected. This lack of a dependence on BM should be considered as the major result of this study and supports results of the other studies mentioned above. Such data add to the evidence that at least above a certain BM threshold, any increase in BM in herbivores will not automatically lead to an increase in digestive efficiency (Clauss et al., 2007; Steuer et al., 2011).

5. Conclusions

- It is possible to quantify the differences in fibre digestion between ruminant foregut fermenters and hindgut fermenters using residual *in vitro* gas production (digestibility) of FNDF. The HGT (96 h) is a useful and quick method to detect also small differences in fibre digestion.
- Regardless of digestion type, BM of an animal has no influence on fibre digestion within the range investigated in this study (> 30 kg). This is in accordance with findings of a lack of influence of BM on digesta mean retention time, a major determinant of fibre digestibility.

Acknowledgements

We thank the whole staff of the safari park Beekse Bergen for the great support, especially Rob van Glabbeek and the teams responsible for the elephants, antelopes and rhinos. We thank also the riding stable Lückerath and the owners of the horses for supporting us with their animals, and Prof. Dr. Dr. h.c. U. Braun from the University of Zurich, and Prof. Dr. M. Kreuzer from the ETH Zurich, for logistical support. This is contribution no. 136 of the DFG

373 Research Unit FOR 533 “The Biology of the Sauropod Dinosaurs: The Evolution of
374 Gigantism”.
375

References

Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., MacPhee, R.D.E., Beck, R.M.D., Grenyer, R., Price, S.A., Vos, R.A., Gittleman, J.L., Purvis, A., 2007. The delayed rise of present-day mammals. Nature 446, 507-512.

Bjorndal, K.A., Bolten, A.B., Moore, J.E., 1990. Digestive fermentation in herbivores: effect of food particle size. Physiol. Zool. 63, 710-721.

Clauss, M., Loehlein, W., Kienzle, E., Wiesner, H., 2003. Studies on feed digestibilities in captive Asian elephants (*Elephas maximus*). J. Anim. Physiol. Anim. Nutr. 87, 160-173.

Clauss, M., Polster, C., Kienzle, E., Wiesner, H., Baumgartner, K., von Houwald, F., Streich, W.J., Dierenfeld, E., 2005. Energy and mineral nutrition and water intake in the captive Indian rhinoceros *(Rhinoceros unicornis)*. Zoo Biol. 24, 1-14.

Clauss, M., Castell, J.C., Kienzle, E., Dierenfeld, E.S., Flach, E.J., Behlert, O., Ortmann, S., Streich, W.J., Hummel, J., Hatt, J.-M., 2006. Digestion coefficients achieved by the black rhinoceros (*Diceros bicornis*), a large browsing hindgut fermenter. J. Anim. Physiol. Anim. Nutr. 90, 325-334.

Clauss, M., Schwarm, A., Ortmann, S., Streich, W.J., Hummel, J., 2007. A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. Comp. Biochem. Physiol. A 148, 249-265.

Clauss, M., Nunn, C., Fritz, J., Hummel , J., 2009. Evidence for a tradeoff between retention time and chewing efficiency in large mammalian herbivores. Comp. Biochem. Physiol. A 154, 376-382.

Demment, M.W., Van Soest, P.J., 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. Am. Nat. 125, 641-672.

Ehle, F.R., Jeraci, J.L., Robertson, J.B., Van Soest, P.J., 1982. The influence of dietary fiber on digestibility, rate of passage and gastrointestinal fermentation in pigs. J. Anim. Sci. 55, 1071-1081.

Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1-15.

Foose, T.J., 1982. Trophic strategies of ruminant versus nonruminant ungulates. PhD, University of Chicago, Michigan.

Fritz, J., Hummel, J., Kienzle, E., Arnold, C., Nunn, C., Clauss, M., 2009. Comparative chewing efficiency in mammalian herbivores. Oikos 118, 1623-1632.

Glindemann, T., Tas, B.M., Wang, C., Alvers, S., Susenbeth, A., 2009. Evaluation of titanium dioxide as an inert marker for estimating faecal excretion in grazing sheep. Anim. Feed Sci. Technol. 152, 186-197.

Iason, G.R., Van Wieren, S.E., 1999. Digestive and ingestive adaptations of mammalian herbivores to low-quality forage. In: Olff, H., Brown, V.K., Drent, R.H. (Eds.), Herbivores: between plants and predators, Blackwell, Oxford, pp. 337-369

Jagger, S., Wiseman, J., Cole, D.J.A., Craigon, J., 1992. Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pig. Br. J. Nutr. 68, 729-739.

Justice, K.E., Smith, F.A., 1992. A model of dietary fiber utilization by small mammalian herbivores, with empirical results for *Neotoma*. Am. Nat. 139, 398-416.

Kavanagh, S., Lynch, P.B., O'Mara, F., Caffrey, P.J., 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. Anim. Feed Sci. Technol. 89, 49-58.

Keys, J.E., Soest, P.J.V., Young, E.P., 1969. Comparative study of the digestibility of forage cellulose and hemicellulose in ruminants and nonruminants. J. Anim. Sci. 29, 11-15.

424 Keys, J.E., Van Soest, P.J., 1970. Digestibility of forages by the meadow vole *(Microtus*
425 *Pennsylvanicus)*. J. Dairy Sci. 53, 1502-1508.
426 Kiefer, B., 2002. Qualität und Verdaulichkeit der vom Breitmaulnashorn (*Ceratotherium s.*
427 *simum*) aufgenommenen Nahrung. PhD Thesis, Tierärztliche Fakultät Ludwig-
428 Maximilians-Universität, München, Germany.
429 Liu, J.X., Susenbeth, A., Südekum, K.-H., 2002. *In vitro* gas production measurements to
430 evaluate interactions between untreated and chemically treated rice straws, grass hay,
431 and mulberry leaves. J. Anim. Sci. 80, 517-524.
432 Maddison, W.P., Maddison, D.R., 2006. Mesquite: a modular system for evolutionary
433 analysis. http://mesquiteproject.org. In:
434 Martins, E., 2004. COMPARE, version 4.6. Computer programs for the statistical analysis of
435 comparative data. Distributed by the author at http://compare.bio.indiana.edu/.
436 Department of Biology, Indiana University, Bloomington IN.
437 Martins, E.P., Hansen, T.F., 1997. Phylogenesis and the comparative method: a general
438 approach to incorporating phylogenetic information into the analysis of interspecific
439 data. Am. Nat. 149, 646-667.
440 Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The
441 estimation of the digestibility and metabolizable energy content of ruminant
442 feedingstuffs from the gas production when they are incubated with rumen liquor in
443 vitro. J. Agric. Sci. 93, 217-222.
444 Menke, K.H., Huss, W., 1987. Tierernährung und Futtermittelkunde, 3rd Ed., Eugen Ulmer,
445 Stuttgart, Germany.
446 Owen-Smith, R.N., 1988. Megaherbivores: the influence of very large body size on ecology.
447 Cambridge University Press, Cambridge, UK.
448 Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature 401, 877-884.
449 Pérez-Barbería, F.J., Elston, D.A., Gordon, I.J., Illius, A.W., 2004. The evolution of
450 phylogenetic differences in the efficiency of digestion in ruminants. Proc. R. Soc. B
451 271, 1081-1090.
452 Prins, R.A., Cliné-Theil, W.C., Van´t Klooster, A.T., 1981. An *in vivo* procedure for the
453 estimation of in vivo digestibility of roughage plant cell wall components in
454 herbivores using mixed rumen microorganisms. Agric. Environ. 6, 183-194.
455 Prins, R.A., Rooymans, T.P., Veldhuizen, M., Domhof, M.A., Cliné-Theil, W., 1983. Extent
456 of plant wall digestion in several species of wild ruminants kept in the Zoo. Zool.
457 Garten 53, 393-403.
458 Robbins, C.T., Spalinger, D.E., Hoven, W.v., 1995. Adaptation of ruminants to browse and
459 grass diets: are anatomical-based browser-grazer interpretations valid? Oecologia 103,
460 208-213.
461 Rohlf, F., 2001. Comparative methods for the analysis of continuous variables: geometric
462 interpretations. Evolution 55, 2143-2160.
463 Smith, L.W., Goering, H.K., Gordon, C.H., 1972. Relationships of forage compositions with
464 rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55, 1140-
465 1147.
466 Steuer, P., Clauss, M., Südekum, K.-H., Hatt, J.-M., Silinski, S., Klomburg, S., Zimmermann,
467 W., Fickel, J., Streich, W.J., Hummel, J., 2010. Comparative investigations on
468 digestion in grazing (*Ceratotherium simum*) and browsing (*Diceros bicornis*)
469 rhinoceroses. Comp. Biochem. Physiol. A 156, 380-388.
470 Steuer, P., Südekum, K.-H., Müller, D.W.H., Franz, R., Kaandorp, J., Clauss, M., Hummel, J.,
471 2011. Is there an influence of body mass on digesta mean retention time in herbivores?
472 A comparative study on ungulates. Comp. Biochem. Physiol. A 160, 355-364.

Titgemeyer, E.C., Armendariz, C.K., Bindel, D.J., Greenwood, R.H., Löest, C.A., 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. J. Anim. Sci. 79, 1059-1063.

Udén, P., Van Soest, P.J., 1982. Comparative digestion of timothy (*Phleum pratense*) fibre by ruminants, equines and rabbits. Br. J. Nutr. 47, 267-272.

Van Soest, P.J., Robertson, J.B., 1985. Analysis of forages and fibrous foods. AS 613 Manual, Department of Animal Sience, Cornell University, Ithaca, NY.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant, 2nd edition. Cornell University Press, Ithaca N.Y.

Van Soest, P.J., Dierenfeld, E.S., Conklin, N.L., 1995. Digestive strategies and limitations of ruminants. In: Engelhardt, W.v., Leonhard-Marek, S., Breves, G., Giesecke, D. (Eds.), Ruminant physiology: digestion, metabolism, growth and reproduction. Proceeding 8th International Symposium on Ruminant Physiology, pp. 581-600

Van Soest, P.J., 1996. Allometry and ecology of feeding behavior and digestive capacity in herbivores: a review. Zoo Biol. 15, 455-479.

van Wieren, S.E., 1996. Digestive strategies in ruminants and nonruminants. Ph.D. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 2007. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Wenninger, P.S., Shipley, L.A., 2000. Harvesting, rumination, digestion, and passage of fruit and leaf diets by a small ruminant, the blue duiker. Oecologia 123, 466-474.

Table 1

Body mass (BM) [kg] of the studied animals and grass hay factors: fibre (NDF, ADF, ADL) and crude protein (CP) content [% OM] and 24 h gas production (GP) [ml/200 mg OM] measured with the Hohenheim gas test (± standard deviation (SD) or both individual values when n = 2)

		n	BM [kg]	NDF	ADF	ADL	CP	24 h GP [ml/200 mg OM]
				[% OM]				
Ruminant foregut fermenters								
Springbok[3]	*(Antidorcas marsupialis)*	2	30* (30/30)	71.0	39.5	4.6	10.4	34.0
Domestic goat[1]	(*Capra aegagrus hircus)*	6	58 ±4.7	76.6	43.1	6.9	7.7	34.9
Domestic sheep[2]	(*Ovis orientalis aries)*	3	94 ±4.2	71.0	39.4	5.7	7.0	36.5
Blue wildebeest[3]	(*Connochaetes taurinus)*	5	160* ±0.0	70.7	39.1	4.1	11.8	34.1
Oryx antelope[3]	*(Oryx gazella)*	3	170* ±17.3	70.7	39.1	4.1	11.8	34.1
Sable antelope[3]	*(Hippotragus niger)*	3	170* ±17.3	74.6	39.5	6.4	11.3	31.7
Waterbuck[3]	(*Kobus ellipsiprymnus)*	2	210* (180/240)	73.4	42.0	7.8	10.9	26.2
Forest buffalo[3]	(*Syncerus caffer nanus)*	2	350* (350/350)	73.4	42.0	7.8	10.9	26.2
Bactrian camel[3]	*(Camelus ferus)*	4	450* ±0.0	71.0	39.5	4.6	10.4	34.0
Domestic cattle[1]	*(Bos primigenius taurus)*	3	1287 ±25.2	73.6	38.9	3.9	9.5	33.7
Mean ±SD				**72.6 ±2.03**	**40.2 ±1.53**	**5.6 ±1.54**	**10.2 ±1.65**	**32.5 ±3.54**
Hindgut fermenters								
Warthog[3]	*(Phacochoerus africanus)*	1	77	75.8	41.6	4.6	12.1	24.8
Shetland pony[2]	(*Equus ferus caballus)*	3	97 ±6.1	71.0	39.4	5.7	7.0	37.4
Przewalski horse[3]	*(Equus ferus przewalskii)*	4	250* ±0.0	71.0	39.5	4.6	9.4	34.0
Grevy´s zebra[3]	(*Equus grevyi)*	4	390* ±20.0	74.6	39.5	6.4	11.3	31.7
Domestic horse[4]	*(Equus ferus caballus)*	6	564 ±49.2	66.9	30.0	3.1	9.5	34.0
White rhinoceros[3]	*(Ceratotherium simum)*	7	1800* ±146.6	64.2	34.3	5.9	11.7	41.8
African elephant[3]	*(Loxodonta africana)*	6	4000* ±1300	71.0	39.5	4.6	10.4	34.0
Mean ±SD				**70.6 ±4.05**	**37.7 ±4.05**	**5.0 ±1.11**	**10.2 ±1.77**	**34.0 ±5.20**
P-value				**0.206**	**0.098**	**0.390**	**0.972**	**0.512**

*Body masses were estimated; [1]University of Bonn, Germany; [2]University and ETH Zurich, Switzerland; [3]Safari Park Beekse Bergen, Netherlands; [4]Riding stable Lückerath, Germany (p-values were calculated between ruminating foregut and hindgut fermenters with the t-test; NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin (fibre fractions were analyzed sequentially, the NDF and ADF were corrected for ash using the insoluble ash after lignin determination), OM = organic matter)

Table 2

Gas production (GP) for faecal NDF residues [ml/200 mg NDF] of the measured intervals (0-8 h, 8-16 h, 16-24 h, 24-48 h, 48-72 h and 72-96 h), cumulative gas production [ml/200 mg NDF] (GP_{cum}) for 96 h of incubation and faecal NDF (FNDF) content [% DM] (± standard deviation (SD) or both individual values when n = 2)

	GP_{0-8} h	**GP_{8-16} h**	**GP_{16-24} h**	**GP_{24-48} h**	**GP_{48-72} h**	**GP_{72-96} h**	**96 h GP_{cum}**	**FNDF**
				[ml/200 mg NDF]				**[% DM]**
Ruminant foregut fermenters								
Springbok	0.99 0.90/1.06	0.00 0.00/0.00	0.18 0.00/0.35	3.31 2.71/3.91	4.20 3.61/4.80	1.88 1.81/1.95	10.29 9.03/11.54	50.9 50.5/51.2
Domestic goat	1.23 ±0.450	0.05 ±0.080	0.40 ±0.266	4.27 ±0.934	4.78 ±0.859	2.68 ±0.248	13.40 ±2.420	64.1 ±2.73
Domestic sheep	0.23 ±0.239	0.67 ±0.657	1.10 ±0.915	6.60 ±3.175	6.53 ±2.145	4.13 ±1.274	18.46 ±7.559	54.8 ±7.20
Blue wildebeest	0.12 ±0.206	0.76 ±0.417	0.88 ±0.420	5.12 ±1.087	4.50 ±0.465	3.42 ±0.574	14.65 ±1.417	52.9 ±3.61
Oryx antelope	0.13 ±0.220	0.17 ±0.140	0.13 ±0.149	3.33 ±0.606	4.50 ±0.732	3.00 ±0.750	11.34 ±0.772	56.1 ±2.71
Sable antelope	0.60 ±0.227	0.43 ±0.248	0.50 ±0.293	4.57 ±0.846	3.93 ±0.209	2.33 ±0.564	12.58 ±1.792	52.7 ±5.40
Waterbuck	0.31 0.45/0.18	0.31 0.36/0.27	1.09 1.45/0.73	5.26 6.88/3.63	4.13 4.53/3.72	2.17 2.62/1.73	13.19 16.21/10.17	48.3 45.0/51.7
Forest buffalo	1.16 0.72/1.60	0.09 0.18/0.00	0.36 0.63/0.09	4.53 4.89/4.16	4.75 4.44/5.06	3.63 2.99/4.27	13.89 13.86/13.92	57.0 56.2/57.7
Bactrian camel	1.03 ±0.601	0.00 ±0.000	0.10 ±0.123	1.45 ±1.230	2.98 ±0.536	2.35 ±0.698	7.94 ±1.277	47.7 ±4.99
Domestic cattle	0.40 ±0.258	0.07 ±0.149	0.00 ±0.000	1.80 ±1.053	3.83 ±0.585	2.40 ±0.540	8.66 ±1.968	56.1 ±3.07
Mean	**0.62 ±0.446**	**0.25 ±0.250**	**0.48 ±0.403**	**4.02 ±1.505**	**4.41 ±0.877**	**2.80 ±0.713**	**12.60 ±3.118**	**54.0 ±4.77**
Hindgut fermenters								
Warthog	1.30	0.50	2.70	15.40	8.10	5.30	33.1	67.1
Shetland pony	1.77 ±0.633	0.43 ±0.628	1.53 ±0.951	10.23 ±1.339	6.90 ±0.769	4.00 ±0.399	24.78 ±2.237	68.7 ±1.82
Przewalski horse	1.06 ±0.729	0.18 ±0.253	1.14 ±0.880	7.50 ±2.881	8.16 ±1.710	6.29 ±0.949	24.42 ±5.618	70.7 ±2.82
Grevy´s zebra	1.28 ±0.479	0.55 ±0.853	1.58 ±0.795	8.35 ±1.724	7.45 ±0.936	6.13 ±1.207	25.31 ±3.056	66.8 ±1.68
Domestic horse	1.32 ±0.320	0.68 ±0.799	2.06 ±1.057	10.30 ±2.220	7.45 ±1.254	5.48 ±1.057	27.29 ±3.696	70.2 ±1.71
White rhinoceros	0.89 ±0.233	0.20 ±0.273	1.59 ±0.681	9.80 ±2.452	7.50 ±2.765	5.79 ±1.258	26.02 ±4.185	55.6 ±6.36
African elephant	0.95 ±0.424	1.10 ±1.257	3.38 ±1.060	8.78 ±1.903	7.47 ±1.830	5.83 ±1.459	27.50 ±2.438	63.8 ±6.56
Mean	**1.21 ±0.296**	**0.52 ±0.315**	**2.00 ±0.789**	**10.07 ±2.564**	**7.57 ±0.424**	**5.55 ±0.772**	**26.92 ±2.980**	**66.1 ±5.16**

(NDF = neutral detergent fibre, DM = dry matter)

Table 3

Results of the statistical analysis of the whole dataset using OLS and PGLS (Dependent variables: GP in the intervals 0-8 h, 8-16 h, 16-24 h, 24-48 h, 48-72 h and 72-96 h; cumulative 96 h GP (GP_{cum}); FNDF; independent variables: BM, DT, co-variable: 24 h GP of the food (GP_{food})). Significant results in bold.

Dependent variables	**Independent variables**	**OLS F**	**p**	**R^2**	**PGLS t**	**p**	**R^2**
$GP_{0 - 8 h}$	BM	1.585	0.230	0.48	1.25	0.233	0.46
	DT	**11.691**	**0.005**		**3.33**	**0.005**	
	24 h GP_{food}	0.180	0.678		0.59	0.625	
$GP_{8 - 16 h}$	BM	0.434	0.521	0.22	0.73	0.475	0.22
	DT	2.090	0.172		1.50	0.156	
	24 h GP_{food}	0.004	0.950		0.00	1.00	
$GP_{16 - 24 h}$	BM	0.733	0.407	0.69	1.62	0.129	0.72
	DT	**23.110**	**<0.001**		**5.15**	**<0.001**	
	24 h GP_{food}	1.712	0.213		2.00	0.067	
$GP_{24 - 48 h}$	BM	2.947	0.110	0.80	1.68	0.117	0.80
	DT	**51.844**	**<0.001**		**7.14**	**<0.001**	
	24 h GP_{food}	1.573	0.232		1.27	0.225	
$GP_{48 - 72 h}$	BM	1.293	0.276	0.84	1.10	0.290	0.84
	DT	**64.059**	**<0.001**		**7.93**	**<0.001**	
	24 h GP_{food}	0.191	0.669		0.40	0.700	
$GP_{72h - 96 h}$	BM	757	0.400	0.80	0.87	0.398	0.80
	DT	**39.952**	**<0.001**		**6.21**	**<0.001**	
	24 h GP_{food}	0.014	0.906		0.00	1.00	
96 h GP_{cum}	BM	0.961	0.345	0.87	0.94	0.363	0.87
	DT	**82.959**	**<0.001**		**9.02**	**<0.001**	
	24 h GP_{food}	0.781	0.393		0.90	0.384	
FNDF	BM	1.909	0.190	0.68	1.29	0.221	0.65
	DT	**26.500**	**<0.001**		**4.93**	**<0.001**	
	24 h GP_{food}	0.006	0.941		0.19	0.850	

(GP = gas production in the Hohenheim gas test, BM = body mass, FNDF = faecal neutral detergent fibre, DT = digestion type (ruminant foregut fermenter or hindgut fermenter), OLS = Ordinary Least Squares, PGLS = Phylogenetic Generalized Least-Squares)

Table 4

Allometric regression analysis ($y = a + BM^b$) for the cumulative 96 h gas production (GP_{cum}) for faecal NDF (BM data was log transformed).

Variable	Group	Statistics	a	CI	b	CI	P	R^2
96 h GP_{cum}	all	OLS	12.0	4.0-35.9	0.06	-0.13-0.25	0.511	0.03
		PGLS	15.5	5.2-45.7	0.02	-0.16-0.20	0.861	0.01
	rum	OLS	21.9	8.4-57.5	-0.11	-0.30-0.07	0.189	0.21
		PGLF	21.9	9.8-49.0	-0.11	-0.27-0.05	0.310	0.20
	hind	OLS	29.1	17.6-48.2	-0.01	-0.10-0.07	0.683	0.04
		PGLS	30.2	20.9-43.7	-0.02	-0.08-0.04	0.549	0.08

(OLS = Ordinary Least Squares, PGLS = Phylogenetic Generalized Least-Squares, CI = Confidence Interval, BM = body mass, NDF = neutral detergent fibre, all = whole dataset, rum = ruminant foregut fermenter, hind = hindgut fermenter)

Table 5

Results of ANOVA on NDF-digestibility from Foose (1982). Significant results in bold.

Forage	Independent Variables	OLS F	p	R^2	PGLS t	p	R^2
Grass hay	BM	2.679	0.117	0.39	1.73	0.099	0.32
	DT	**11.028**	**0.003**		**2.78**	**0.011**	
	ADL	0.042	0.840		0.13	0.899	
Lucerne hay	BM	0.477	0.498	0.61	0.57	0.574	0.62
	DT	3.822	0.065		1.84	0.081	
	ADL	**26.971**	**<0.001**		**5.33**	**<0.001**	

(NDF = neutral detergent fibre, BM = body mass, DT = digestion type (ruminant foregut fermenter or hindgut fermenter), ADL = acid detergent lignin, OLS = Ordinary Least Squares, PGLS = Phylogenetic Generalized Least-Squares)

Table 6

Studies on the relation between body mass (BM) and neutral detergent fibre (NDF) digestibility, the used dataset, animal species included in the studies and the results of the comparison

Source	Data base	Animals	Species	Results
Owen Smith (1988), Clauss et al. (2009)	Foose (1982)	ungulates	36	no influence of BM on NDF digestibility
Wenninger and Shipley (2000), Pérez-Barbería et al. (2004)	Foose (1982) + various sources	ruminants	63	no influence of BM on NDF digestibility
Van Soest et al. (1995), Van Soest et al. (1996)	Keys et al. (1969), Keys and Van Soest (1970), Ehle et al. (1982), Foose (1982), Udén and Van Soest (1982)	ungulates, lagomorphs, rodents	40	influence of BM on cellulose digestibility in grass, no influence on cellulose digestibility in lucerne and on hemicellulose digestibility in both forages
Justice and Smith (1992)	Justice and Smith (1992)	rodents	4	no influence of BM on NDF digestibility

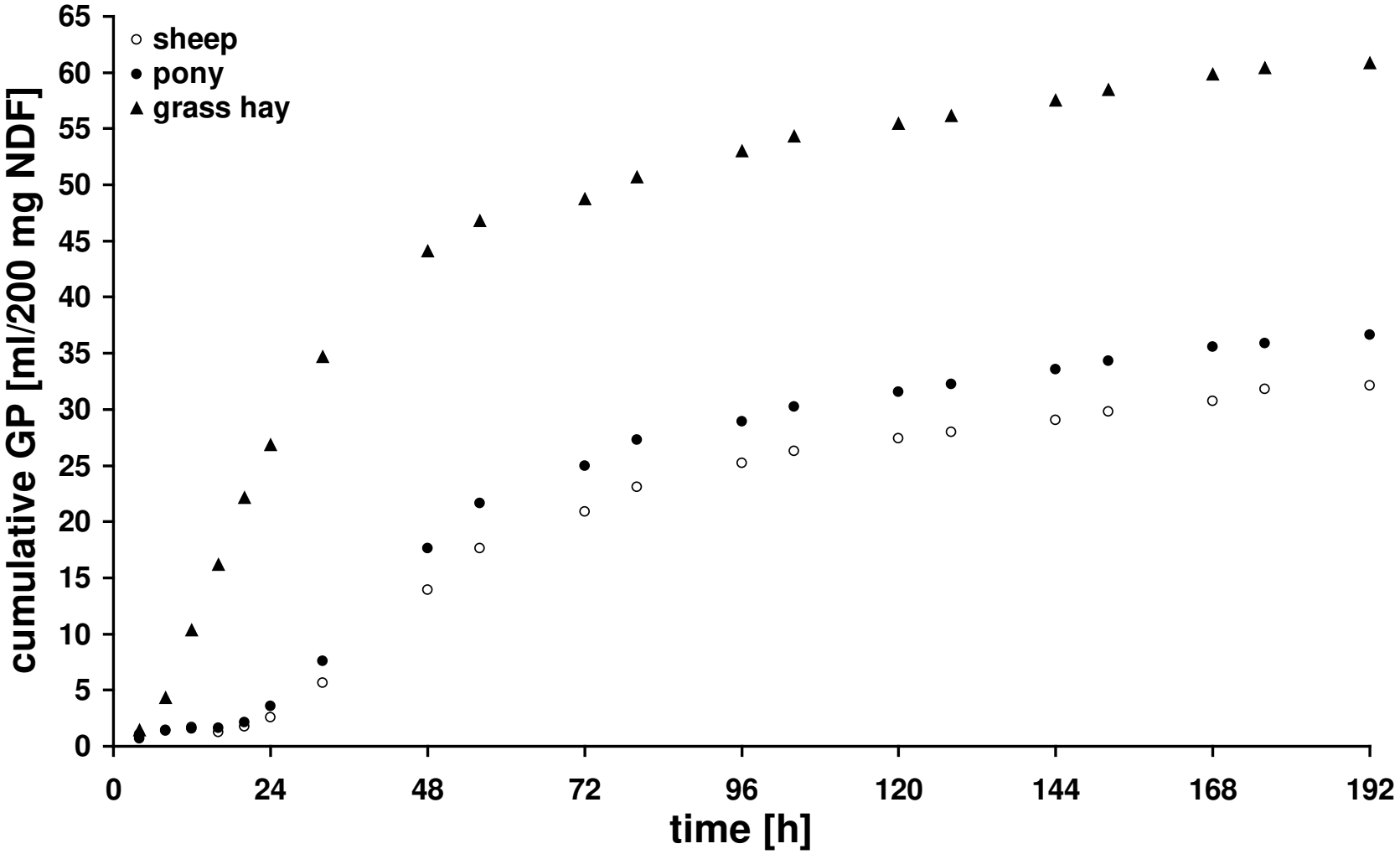

Fig. 1. The cumulative gas production (GP) [ml/200 mg NDF] during the incubation of 192 h for neutral detergent fibre (NDF) of sheep and pony faeces and grass hay (NDF contents were corrected for ash using the insoluble ash after NDF determination)

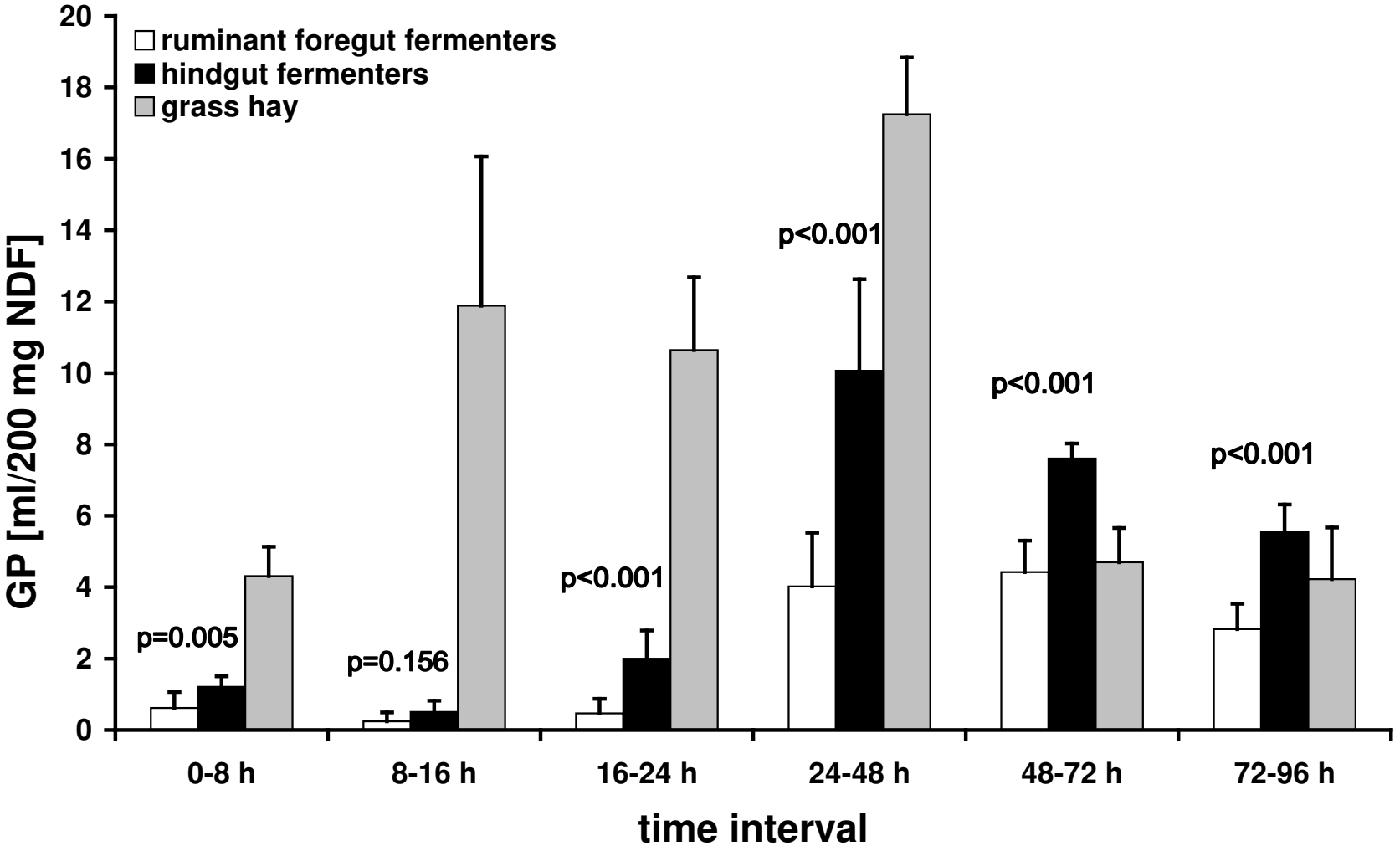

Fig. 2. The gas production (GP) [ml/200 mg NDF] per time interval after incubation for neutral detergent fibre (NDF) of ruminant foregut fermenter and hindgut fermenter faeces and grass hay (p-values between ruminating foregut and hindgut fermenters using PGLS (Table 3), NDF contents were corrected for ash using the insoluble ash after NDF determination)